Creosote P1/P13 blend: 13-Week Inhalation Study in Rats
Creosote Council II. 1995. MRID No. 43601001

EPA Reviewer: Tim McMahon, Ph.D. _____ Date: _____
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Data Evaluation Record

Title: R. I. Hilaski; March 28, 1995; Thirteen week subchronic inhalation toxicity study on North American P1/P13 Creosote CTM in rats.; International Research and Development Corp., Mattawan, MI 49071; Project No. 671-016; Creosote Council II; MRID # 43601001; Unpublished

Performing Lab: IRDC, Mattawan, MI

Test Animal: One hundred seven (107) male and one hundred seven (107) female Sprague-Dawley Crl:CD®BR VAF/PLUS® derived rats were received from Charles River Laboratories, Portage, Michigan July 27, 1993. Of these animals 80 males (181-211 g) and 80 females (130-149 g) were selected on the basis of appropriate body weight, absence of pre-existing ocular lesions and apparent good health. Five additional animals/sex were also selected for a pretest health screen to assure suitability. Animals were assigned at random to the control and treatment groups. Animals from each group assigned to the recovery interval were selected at random.

Test Material: The test material was received February 12, 1992 from H.A. Kremer and Associates (Ontario) Ltd. Bolton, Canada. The material was identified as North American P1/P13 Creosote CTM and described as being black distilled coal tar with an odorous characteristic. It was stored in a cool, dry, ventilated area and was said to be stable through to the end of the study.

Dose: A two-week range-finding inhalation study using P1/P13 (IRDC Study No. 671-015) was conducted on 4 groups of 10 animals/sex. Using the whole body exposure method , rats were exposed to atmospheres containing 0, 23, 96 and 205 mg/m^3 of test material for 6 hours/day and for 5 days/week. No deaths occurred during the treatment period. At terminal sacrifice treatment-related findings included significantly decreased mean terminal body weights (9-15%) of all animals of the mid and high dose groups, as well as, higher relative liver weights in mid and high dose males when compared with control group values. Based on the results of this study it was recommended that the highest dose of P1/P13 for the 13 week inhalation study be set at 100 mg/m^3.

The doses of P1/P13 selected for the definitive inhalation study were 0, 5.4, 49 and 106 mg/m^3 air and these dose groups where referred to as Groups I to IV, respectively, in the study report.

Husbandry: All animals were identified by cage, group, sex and individually by a metal ear tag bearing the animal number. Animals were housed individually and were acclimated for two weeks. They were maintained in environmentally controlled chambers with a 12 hour light/dark cycle. During acclimation, exposure and recovery periods temperature and humidity were within an acceptable range. The only large deviation appeared to be during exposure when temperature and humidity ranged from 17 - 26 °C and 24 - 79 %, respectively. Water and diet (Rodent Chow® No. 5002, Purina Mills) were available *ad libitum*. All animals were housed in their respective chambers 24 hours per day during the 13 week exposure period except during cleaning. Chamber ventilation air was provided by an HVAC system and chamber exhaust air was filtered through a HEPA filter to remove particulates. Chamber airflow, temperature and relative humidity were recorded at one hour intervals during each days exposure and every three hours until midnight during non-exposure hours. The animals were removed from the chambers prior to exposure so that the chambers could be cleaned of excreta and feed jars could be covered. The animals were removed for cleaning of chambers after exposure and feed was made available upon their return. Water was made available only during non-exposure times. Cage position was systematically rotated on a weekly basis.

Treatment Methods: The animals were treated for 6 hours/day, 5 days/week for 13 weeks. Twenty animals/sex/dose were exposed to the desired concentrations. Exposure was carried out in a 6 m^3 (6000 litre) whole-body exposure chamber. The aerosol atmospheres of the test material were generated by having warmed and stirred material metered at a constant rate to an atomizer operated with in-house compressed air. The air and test material produced a concentrated aerosol/vapour in a 4 litre glass atomization chamber. Additional air passing through another inlet purged the aerosol/vapour into the chamber inlet where the chamber-supply-air reduced the concentration to the desired level.

Exposure Parameters:

Atmosphere Generation Settings				
Group Number:	**I**	**II**	**III**	**IV**
Desired Conc. (mg/m^3):	0	5	50	100
Approx. Liquid Flow Rate (ml/min):	-	0.14	0.53	2.5*
Purge Airflow (L/min):	-	4	40	40
Atomizer Pressure (psig):	-	10	30	30
Chamber Airflow (L/min):	1350	1350	1350	1350

* Note an increase of five fold to attain a concentration only 2x that of Group III

Nominal Concentration: Nominal exposure concentrations were determined by taking the weight of the test material reservoirs before and after each exposure generation. The total volume of air passing through the chamber during the exposure was determined by taking an arithmetic mean of the flow rate measurements and multiplying by the exposure duration. Finally, the total amount of material used was divided by the total volume of air passed through the chamber during exposure to give a calculated nominal concentration in mg/m^3.

Actual Concentration: Actual concentrations were measured by standard gravimetric methods. Two samples were collected on 25 mm glass-fiber filter pads from each treatment group chamber during each six hour exposure. The sampling period was approximately two and one-half (2.5) hours or about half the treatment period so that two samples represent the concentration over the full day's exposure. Total volume sampled was measured with a dry gas meter. The difference in the pad pre- and post-sampling weights was calculated and divided by the sample volume.

Aerosol Particle Size Determination: Determined once each exposure day for each group. The samples were collected using a cascade impactor operated at a flow rate of 28.3 L/min for an appropriate duration. Net weight on each filter pad was determined and computer analysis using cumulative percentages helped derive aerodynamic diameters smaller than the cutoff. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were generated by computer and calculated.

Chamber Distribution Evaluations: Prior to the first day of exposure the homogeneity of test material distribution was determined for each exposure chamber by comparing five locations to a reference location (routine sampling location). Homogeneity of the exposure appeared to be within acceptable (± 4-13 %) limits in comparison to the reference location.

Evaluation Parameters:

In-Life Examinations: Observations for mortality, morbidity or reaction to treatment were made daily. Thorough clinical examinations, body weight and feed consumption determinations were made weekly. Ophthalmoscopic examinations of the cornea, conjunctiva, sclera, iris and fundus were performed at pretest and prior to terminal or recovery sacrifice. Clinical laboratory tests were conducted on 5 animals/sex at pretest, 10 animals/sex/group at study termination and on all surviving animals at the end of the recovery period. Blood samples were obtained from the orbital sinus following an overnight fasting period.
Hematological parameters assessed: leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocytes, platelet count, differential leukocyte count.
Biochemical parameters assessed: concentrations of sodium, potassium, chloride, calcium, inorganic phosphorous, total bilirubin, urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, serum cholesterol, glucose, and activities of alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferse (ALT), creatinine phosphokinase (CPK), ornithine carbamoyltransferase (OCT), gamma glutamyltranspeptidase (GGT).

Necropsy: Any animals found dead during the course of the study were necropsied. At the terminal and recovery sacrifice all animals were euthanized by intraperitoneal injections of sodium pentobarbital. All animals were subjected to a complete postmortem examination. The following organ weights were determined: adrenals, brain. ovary/testis, heart, kidney, liver, lungs, mammary gland, thymus, thyroid/parathyroid.

Histopathological Examination: Representative sections of the following organs and tissues were collected from all Group 1 and Group IV animals designated for sacrifice after 13 weeks, as well as from any animals that died during the course of the study: adrenals, aorta, bone, bone marrow, bone marrow smear, brain, eye, optic nerve, GI tract, ovary/testis, heart, kidney, lacrimal gland, liver, lung, lymph nodes, mammary glands, nasal tissues, pancreas, pituitary, prostate and seminal vesicles, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, sternum, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, cervix, vagina and all gross lesions.

In addition, sections of the bone, eye, optic nerve, kidney, lung, nasal tissues, spleen, thyroid, heart and trachea were examined microscopically for all animals in Groups II and III at the 13-week terminal sacrifice and all animals in all groups at the recovery sacrifice. A four-step grading system of trace, mild, moderate and severe was used to define gradable lesions for comparison between dosage groups.

Statistics: Non-parametric analysis was conducted when animal numbers in any one group were equal to or less than ten with the Kruskal-Wallis one-way analysis of variance followed by the Mann-Whitney U test where appropriate. On data where the number of animals were greater than ten and the measurements were at least an interval scale, parametric analysis was performed using Bartlett's chi-square test for homogeneity of variance followed by an analysis of variance and where appropriate by Dunnett's t-test. The level of rejection was at the five percent level in all cases.

RESULTS:

Exposure Conditions: As shown in Table I, the weekly actual mean dosing concentration of the treated groups was within an acceptable degree of variability.

Table I Thirteen Week Mean Dose Concentrations

Group Number	Exposure Period (wks)	Times Daily Limit Exceeded	Desired Conc. (mg/m^3)	Actual Mean (Gravimetric) ($mg/m^3 \pm$ S.D.)	Nominal Mean ($mg/m^3 \pm$ S.D.)
II	13	16	5	5.4 ± 0.31	22 ± 1.1
III	13	15	50	49 ± 3.9	128 ± 10.6
IV	13	10	100	106 ± 5.8	221 ± 13.9

The aerosol size distribution along with the cumulative weight percent for particle size lower than 1.05 µm are seen in Table II. Weekly MMAD values did not vary significantly and the GSD were similar in all cases. The cumulative weight percentage of particles below 1.05 µm was 6.13, 12.7 and 9.86 for groups II, III and IV respectively. The dose Group II was significantly lower compared to dose Group III but not from Group IV. However, the MMAD was within acceptable values of 1 - 4 µm in size.

Table II Mean Particle Size Distribution and Cumulative Weight Percent

Group No.	No. of Days	Aerosol Size MMAD	GSD	Cumulative Weight Percent below 1.05 µm	Cumulative Weight Percent below 4.70 µm
II	65	3.0	1.92	6.13	76.1
III	65	2.2	1.99	12.7	86.2
IV	65	2.4	1.91	9.86	86.4

Aerosol analysis revealed low overall mean concentration of nine components measured. About 15 % of the test material readily vaporized under the experimental conditions. Table III identifies the most noted vapour components. Aerosol was not included in overall assessment.

Table III Vapour Components of Chamber Atmosphere

Component	Overall Group Mean (mg/volume of air sampled)		
	II	III	IV
Benzene	3.1	27.5	66.3
Toluene	44.8	130.5	228.8
Ethylbenzene	13.6	69.7	120.3
m + p xylene	29.8	154.3	269.3
o-xylene	13.8	73.1	113.7
cumene	3.2	13.3	19.6
Naphthalene	164.1	542.0	357.3

Component	Overall Group Mean (mg/volume of air sampled)		
2-methylnaphthalene	38.2	75.6	54.2
1-methylnaphthalene	15.3	31.5	22.9
Total	312.4	1090.6	1232.8

Volume of air analyzed varied for each group with an average of 469, 315 and 248 L of air for groups II, III and IV, respectively.

Vapours, analyzed after being captured on a charcoal sorbent tube, consisted of light volatile compounds. Some components appeared to show a high degree of variability between the monthly analysis for the respective doses. Naphthalene was a component which had the widest variability where concentrations ranged from 2.7 - 332 µg, 217 - 781 µg and 105 - 492 µg in Groups II, III and IV respectively. While the report indicated that the analytical means and standard deviations were listed in Tables 5 and 6 of the report, only the mean values were present.

Mortality: One animal died (Group III male) during the exposure phase of the study. This animal was found dead on day 23 of the exposure period. Postmortem examination of this animal determined the cause of death to be the result of cardiac myopathy.

Clinical Signs: The most commonly noted clinical observation in all groups was that of staining of various body areas although the discolouration was not identified. This observation was noted with greater frequency in both male and female rats of Groups III and IV and extended into the 6-week recovery period. Other infrequent clinical observations made in all dose groups included malocclusions, alopecia and scabbed areas on the skin which were considered incidental in nature and not related to treatment.

Body Weight: Exposure Period (weeks 1-13):
Terminal mean body weights As shown in the table below, Group III and IV animals' mean body weights were significantly decreased at weeks 7 and 13. In male rats of Group IV, group mean body weight was decreased significantly for the whole 13 week exposure period, while in female rats of Group IV, group mean body weights were decreased during the whole 13 week exposure period except weeks 3, 5, and 12. The decreases in group mean body weight for male rats (8-9% decreasedvs. Contol) were on average slightly higher than in female rats (5-7% decreased vs control). Terminal mean body weights of Group II (-2.7%) and III (-4.4%) females and Group II (-0.9%) and Group III (-3.4%) males were comparable to control animal body weights.

Table IV: Group Mean Body Weights (grams) in Creosote-exposed Rats

Study week (n = 20)	Dose Group (mg/cubic m.)			
	0	5.4	49	106
Males				
week 1	269±11.9	273±11.9	263±14.1	248±13.4**

week 7	411±28.2	409±23.4	396±30.8	377±26.3**
week 13	466±36.9	462±28.9	450±38.6	428±32.9**
Females				
week 1	174±7.9	176±9.6	170±9.4	165±12.9*
week 7	248±15.3	244±17.1	237±13.7	232±13.2**
week 13	273±19.1	266±19.6	261±18.1	255±18.8**

data taken from page 73 of the report. *p < 0.05 or **p < 0.01 vs. control.

Mean body weight gains of Group IV males and females were -15.2% and -9.6%, respectively, of control values. Group II and III females gained 9.2% and 11.3% less weight, respectively, than control group females over the course of 13 weeks. (See table below). The decreased weight gain observed in Group II females is not considered to be biologically significant as there is less than a 10% difference when compared with control group values and their terminal mean body weights were not significantly different from control animals body weights. In addition, the range-finding study with P1/P13 showed that Group II females gained only 5.7% less weight than controls after having been exposed to more than 3x the concentration of the test material. The decreased mean body weight gain (approx. 5%) of Group II and III males is not considered to be toxicologically significant.

Recovery Period (weeks 14-19): By the end of the six week recovery period the terminal mean body weights of all groups were lower than the control group weights. However, the differences were not significant.

	%Mean Body Weight Change					
Study Weeks	**Group II**		**Group III**		**Group IV**	
	♂	♀	♂	♀	♂	♀
1 - 13	-5.6	-9.2	-5.4	-11.3	-15.2	-9.6
14 - 19	-4.6	-1.0	-1.8	-2.4	+2.5	+1.9

1 - 13 exposure, 14 - 19 recovery

Food Consumption: During the first week of treatment there was a significant decrease ($p<0.01$) in the mean food consumption (g/animal/day) of rats of all treatment groups (13.5, 14.2 and 20.2 % in the male Groups II, III and IV; 14.0, 17.4 and 16.9 % in the female Groups II, III and IV) when compared with food consumption of control groups. For the remainder of the exposure and recovery periods, food consumption was comparable among all groups.

Table IV: Group Mean Food Consumption (g/kg/day) in Creosote-exposed Rats

Study week (n = 20)	Dose Group (mg/cubic m.)			
	0	5.4	49	106
Males				
week 1	86.8±5.0	73.9±5.29**	72.4±3.26**	74.8±16.44*
week 7	58.9±3.18	60.4±3.31	61.4±2.31*	62.5±2.93**
week 13	48.9±3.28	48.7±2.29	48.8±2.32	50.0±2.88

Females

week 1	98.7±5.33	83.7±3.36**	83.5±6.62**	87.6±9.32**
week 7	77.8±13.5	74.9±7.61	78.8±10.08	82.7±21.05
week 13	61.6±5.32	60.1±3.97	62.5±3.45	61.7±9.07

data taken from page 77 and 80 of the report. *p < 0.05 or **p < 0.01 vs. control.

Ophthalmology: No test article-related ophthalmoscopic abnormalities were detected.

Clinical Pathology:

Hematology - Terminal Examination As shown in the table below, Group II animals had a slight increase in the incidence of anisocytosis (male), and an increase in the incidence and severity of poikilocytosis (male and female). Group III males and females had an increased incidence and severity of polychromasia, poikilocytosis and anisocytosis in addition to decreased haemoglobin and haematocrit values (statistical significance was observed in the males only). Group IV male and female animals had decreased hemoglobin, haematocrit and erythrocyte values, increased reticulocyte values (significant only for females), mild (5-25 % cells affected) to moderate (26-50 % cells affected) polychromasia and poikilocytosis and mild anisocytosis.

Hematology Observations (N=10)

	Group I		Group II		Group III		Group IV	
Parameter	♂	♀	♂	♀	♂	♀	♂	♀
Hemoglobin (g/dl)	15.5	15.4	15.4	15.3	**14.3**[2]	14.6	14.6	**13.5**[2]
Hematocrit %	44.7	41.4	43.5	40.9	**39.7**[1]	37.8	40.3	**35.0**[2]
Erythrocyte ($x10^6/mm^3$)	7.77	7.00	7.57	7.04	7.04	6.45	6.99	**5.98**[1]
Reticulocyte (/100 RBC)	2.8	2.9	2.8	3.0	4.0	3.4	5.2	**7.8**[1]
Mild Polychromasia	1/10	1/10	1/10	1/10	2/10	4/10	6/10	5/10
Mild Poikilocytosis	4/10	3/10	9/10	6/10	9/10	6/10	10/10	9/10
Anisocytosis	0/10	2/10	4/10	2/10	6/10	4/10	6/10	8/10

[1]Significantly different from control group; p<0.05
[2]Significantly different from control group; p<0.01

Recovery Examination After the recovery period only a mild increase in anisocytosis of the erythrocytes of some Group III and IV animals was noted.

Serum Biochemistry:

Terminal Examination (See table below) Elevated **cholesterol** levels were observed in both sexes of Groups III and IV animals (significance in Group III males and Group III and IV females) although they fell below the range of pretest levels (122/106 mg/dl for males and females, respectively). Exposure to atmospheres of P1/P13 resulted in elevated cholesterol levels which may be related to the decreased body weights and/or the mild histopathological thyroid gland changes, since serum cholesterol levels may vary with thyroid gland activity. The changes observed in **ALT** and **GGT** of Groups III and IV were within the range of the lab's historical control values and were considered normal biological variation.

Increased serum **phosphorous levels** were observed in all treated males with the difference achieving statistical significance only in Group IV males. However, these phosphorous levels were comparable to those levels obtained at pretest examination. All other Group II biochemical parameters were comparable to control group

values.

Parameter	Biochemical Parameter Observations (N=10)							
	Group I		Group II		Group III		Group IV	
	♂	♀	♂	♀	♂	♀	♂	♀
Phosphorus (mg/dl)	7.6	7.3	8.9	6.9	8.3	7.3	**8.5**[2]	7.0
Cholesterol (mg/dl)	52	77	52	76	**74**[1]	**109**[2]	70	**116**[2]
Alanine Aminotrans (U/L)	34	35	28	26	28	28	**25**[1]	30
Gamma GlutamylT (U/L)	1	1	4	1	1	**3**[1]	2	**4**[1]

[1]Significantly different from control group; $p<0.05$
[2]Significantly different from control group; $p<0.01$

Recovery Examination All treatment group parameters were comparable to control group levels except for the significantly elevated phosphorous levels in Group III and IV females (7.1 and 7.2 mg/dl, respectively, cf 5.9 mg/dl in control females, $p<0.05$). Recovery levels of phosphorous in Group II and IV females approximated those determined at the pretest examination (10.5 mg/dl). This finding has unknown biological significance in this study and the authors did not offer any explanation.

Macroscopic Pathology:

Terminal Examination No treatment-related observations were noted in either sex of Group II animals. A diffuse grey discolouration of the lungs was observed in Group III (8/10 males & 10/10 females) and Group IV (10/10 male & 10/10 female) animals, all of which were exposure-related to the deposition of the test material.
Recovery Examination Focal or diffuse tan discolouration of the lungs in Group III (2/9 male & 2/10 female) and Group IV (5/10 male & 1/10 female) animals was still evident.

Organ Weights:

Terminal Examination (See table below)

Liver

Group III males and **Group IV** females showed an increased absolute liver weight while both sexes in these groups showed a significant increase in liver/body weight ratios. A significant increase in the liver/brain weight ratio was observed in the females of both groups and a positive trend was observed in the male liver/brain weight ratio of these two groups. The toxicologic significance of these treatment-related increases is uncertain due to the lack of macroscopic or microscopic observations to corroborate these findings.

Adrenal

Male adrenal/body weight ratios of **Groups III and IV** were elevated in the terminal examination (significant). An increase in the adrenal/brain weight ratio was not statistically significant. Furthermore, there were no associated macroscopic or microscopic pathological changes observed in the adrenals.

Lung/trachea

A statistically significant increase in the lung/trachea/body weight ratio of the **Group IV** male and female rats was observed and was likely due to the deposition of the test material in the lungs since a tan discolouration of the lungs was observed macroscopically and pigmented macrophages in the lung were identified histologically. In the absence of any corroborating tissue histopathology, the increases in lung and adrenal weights and weight ratios of animals in Group III and IV may be attributed to the observed decreases of their terminal group mean body weights when compared with those of control animals as reported above in the **Body Weight** section.

Thyroid

Absolute and relative thyroid weights were comparable among all groups.

Absolute and relative organ weights of **Group II** rats were comparable to those of control group animals.

Recovery Examination There were no treatment-related changes in organ weight parameters observed after the recovery period.

Mean Organ Weight Values

	Group I		Group II		Group III		Group IV	
EXPOSURE	♂	♀	♂	♀	♂	♀	♂	♀
Heart (g)	1.37	0.87	1.43	0.93	1.35	0.93	1.39	0.95
Brain (g)	1.99	1.85	2.00	1.84	2.06	1.84	1.97	1.84
Liver (g)	11.66	6.95	12.16	6.88	**13.62**[1]	7.78	13.37	**8.18**[2]
Liver/Body Wt. (%)	2.66	2.83	2.86	2.89	**3.19**[2]	**3.24**[2]	**3.45**[2]	**3.58**[2]
Liver/Brain Wt. (%x10^{-2})	5.88	3.76	6.10	3.75	6.65	**4.23**[1]	6.82	**4.45**[2]
Lung/Trachea (g)	1.61	1.17	1.61	1.13	1.73	1.22	1.66	1.33
Lung/Trachea/Body Wt. (%x10)	3.68	4.76	3.80	4.76	4.07	5.12	**4.31**[2]	**5.89**[1]
Lung/Trachea/Brain Wt. (%x10^{-1})	8.10	6.33	8.04	6.14	8.46	6.66	8.45	7.30
Adrenal (mg)	56	69	54	68	67	71	61	70
Adrenal/Body Wt. (%x10^{3})	12.7	28.2	12.9	28.5	**15.7**[2]	29.5	**15.9**[1]	30.7
Adrenal/Brain Wt. (%)	2.81	3.75	2.71	3.70	3.25	3.83	3.12	3.85
RECOVERY								
Adrenal (mg)	57	79	58	74	56	66[1]	65	79
Adrenal/Body Wt. (%x10^{3})	11.7	28.9	12.0	26.3	11.8	25.0	13.8	30.0

[1]Significantly different from control group; $p<0.05$
[2]Significantly different from control group; $p<0.01$

Microscopic Pathology:
Terminal Examination

Heart Diffuse myocardial degeneration affecting mainly the right side of the heart was observed in the one male of **Group III** which died during the exposure phase and in one male and one female of **Group IV**. Arterial medial hypertrophy of small arterioles in the lung, brown pigment within the convoluted tubules of the kidney, alveolar macrophages containing brown pigment consistent with hemosiderin within the lung and diffuse centrilobular fibrosis within the liver were also observed in the Group III male that died. It was suggested that the cardiac lesion of the animal that died during the study was of sufficient severity to have caused its death.

Alveolar macrophages Small trace levels of black pigment granules within alveolar macrophages were observed in all animals in **Groups II, III and IV**. These macrophages were uniformly dispersed. Distribution was considered multifocal in 8/10 males and all females of Group II and diffuse in 2/10 males of Group II and all animals of Group III and IV. This was considered to be treatment- related by the author.
Nasal tissues The occurrence of small cystic spaces containing basophilic mucoid material in the olfactory epithelium at all four levels of the nasal tissues, in both sexes of **Groups III and IV** and in some females of **Group II** was considered to be exposure- related. The following table illustrates the distribution of the changes noted. Level A represents the most outer portion of the nasal passage. The incidence of the cysts lessened progressively from nasal tissue A to nasal tissue D.

Incidence of Mucoid Cysts of the Olfactory Epithelium in the Nasal Tissue A, B, C and D

Group	I		II		III		IV	
Sex	M	F	M	F	M	F	M	F
Number Examined	10	10	10	10	10	10	10	10
Nasal Tissue A				4	5	5	1	4
Nasal Tissue B				1	6	8	6	9
Nasal Tissue C				1	6	3	5	7
Nasal Tissue D					1		3	2

Thyroid An increase in the incidence of thyroid follicular cell hypertrophy, which resulted in a reduced amount of colloid present within thyroid follicles, was observed in 3/10, 7/10, 8/11 and 8/10 males and 0/10, 0/10, 1/9 and 8/10 females of Groups I, II, III and IV, respectively. Significant increased incidences were observed, therefore, in **Group II, III and IV** males and **Group IV** females. The severity of the effect was characterized as being trace in all affected animals.

No treatment-related histological changes were noted in any other tissues/organs of any animal groups.

Recovery Examination

Alveolar macrophages Small black pigment granules within alveolar macrophages were observed in male and female animals of all treated groups. Macrophages were detected in all lobes of the lungs indicating a uniform dispersion. Multifocal distribution was observed in **Group II and III** males (10/10 and 9/9, respectively) and **Group II, III and IV** females (10/10, 10/10 and 2/10, respectively) while distribution was considered diffuse in **Group IV** males and females (10/10 and 8/10, respectively).

Nasal tissues Small cystic spaces in the olfactory epithelium, usually containing basophilic mucoid material, were present at all levels of the nasal tissue in male and female animals of **Groups III and IV**. Test article-related mucoid cysts were present in several Group III and IV male and female animals. The occurrence of these cysts is shown in the table below.

Incidence of Mucoid Cysts of the Olfactory Epithelium in the Nasal Tissue A, B, C and D

Group	I		II		III		IV	
Sex	M	F	M	F	M	F	M	F
Number Examined	10	10	10	10	9	10	10	10
Nasal Tissue A		1			3	1	5	4
Nasal Tissue B					4	4	9	6
Nasal Tissue C					1		4	3
Nasal Tissue D					1		1	

Thyroid Trace follicular epithelial hypertrophy was observed in both sexes of **All** treatment groups including controls (10/10, 9/10, 8/9 and 10/10 in males and 3/10, 2/10, 0/10 and 4/10 in females in **Groups I, II, III and IV**, respectively). No treatment related increased incidence was observed when compared with control animals by the end of the recovery period. Further investigation revealed that the incidence was upgraded from **no incidence** to **trace** in all animals by the peer review pathologist. Clearly, a difference of interpretive opinions in severity of hypertrophy was the single most important factor used to characterize this as a treatment related effect. Due to the subjective nature of this parameter and since the severity noted was border line as well as questionable among reviewing pathologists the follicular cell hypertrophy was dismissed as being test article

related.

A peer review of the unaudited draft of histopathology data was conducted by Suzanne Botts, D.V.M., Ph.D., A.C.V.P. Diplomate of Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina.

Author's Conclusion:
Exposing groups of male and female rats to aerosol concentrations of 5.4, 49 and 106 mg/m^3 of North American P1/P13 creosote CTM for 13 weeks (6 hours/day, 5 days/week) produced several treatment-related changes, primarily at the two highest exposure levels. By the end of the recovery sacrifice (6 week post exposure) most exposure-related effects were not evident. Long term toxicological significance of the treatment related effects at these doses cannot be determined from this study.

No observable adverse effects were produced in rats following exposure to P1/P13 creosote CTM at a concentration of 5.4 mg/m^3 (0.0054 mg/L) for 13 weeks.

Evaluator's Comments:
The study protocol and report are acceptable and I concur with the author's selection of a NOAEL of 5.4 mg/m^3. The following observations of treatment-related toxicity were reported in the mid (49 mg/m^3) and high (106 mg/m^3) dose animal groups (Group III and IV, respectively) at the termination of the exposure period:

1. Decreased terminal mean body weights (Group IV- **m/f**) and mean body weight gain (Group III - **f**, Group IV - **m/f**).
2. Changes in hematological parameters indicating possible anaemia (decreases in numbers of circulating erythrocytes, % hemoglobin and hematocrit) and effects on hemopoeitic system (increased number of circulating reticulocytes, altered erythrocyte morphology as indicated by presence of poikilocytosis and anisocytosis) (Group III - **m** and Group IV - **f**).
3. Changes in serum biochemistry - increased cholesterol (Group III - **m/f** , Group IV - **f**) and phosphorous levels (Group IV - **m**).
4. Increased incidence of small cystic spaces in nasal cavity epithelium (Group III & IV - **m/f**).
5. Significant increases in liver weights (Group III - **m** and Group IV - **f**), liver/body weight ratios (Group III & IV - **m/f**) and liver/brain weight ratios (Group III & IV - **f**).
6. Increased lung/trachea/body weight ratios, macroscopic tan discoloration of lung tissue and microscopic presence of black pigment granules in alveolar macrophages (Group IV - **m/f**).

Myocardial degeneration was noted in one mid dose male that died during the exposure period and in two high dose rats (one male and one female) at terminal sacrifice. Since cardiac pathology was noted to some degree in animals of all groups, including controls, it is possible that treatment with creosote may have exacerbated a pre-existing pathological condition in these animals.

Animals of the low dose group (5.4 mg/m^3) exhibited: occasional cysts in the nasal cavity epithelium (females only), the presence of pigmented alveolar macrophages (males and females) and increased incidences of mild poikilocytosis and anisocytosis (females only). The hematological effects were not observed after the 6-week recovery period.

After the six week recovery period, increased levels of serum phosphorous in high dose females, presence of small cysts in the nasal cavity of mid and high dose animals (although at lower incidence) and pigmented alveolar macrophages observed in the lung tissues of all treated groups were the only treatment-related changes observed. Terminal body weights were comparable among all groups and there were no differences in any organ weight parameters.

Although treatment-related changes in the hematological parameters were largely resolved by the end of the recovery period, the long term toxicological significance of these findings (anaemia and alterations of haemopoiesis) cannot be determined from this study particularly as the treatment protocol (5 days/week) allows for a 2 day recovery period throughout the exposure period.

Executive Summary:

In a subchronic inhalation toxicity study with P1/P13 creosote (MRID # 43601001), 20 Sprague-Dawley rats/sex/group were treated for thirteen weeks, five days a week, six hours per day with P1/P13 Creosote CTM via whole body exposure at doses of 0, 5.4, 49 and 106 mg/m^3 (0.005, 0.049 and 0.106 mg/L in air, respectively) measured gravimetrically. The aerosol MMAD was between 2.2 and 3.0 microns with a geometric standard deviation between 1.91 and 1.99. Subsequent to the exposure period 10 animals/sex/group were allowed to recover for 6 weeks.

During the study one male rat of the mid dose group (49 mg/m^3) died from myocardial degeneration that resulted in heart failure. One male and one female rat in the highest dose group had similar lesions observed at terminal necropsy. Cardiac pathology (ie: hemorrhage, lymphocytic infiltration and cardiomyopathy) was noted in all animals of all groups (including controls) and this condition may have been exacerbated by treatment with creosote in the mid and high dose animals. Significant treatment-related findings in the mid and high dose animals after the exposure period included decreased body weight gains of both sexes (resolved by the end of recovery period), altered hematological parameters (decreased hemoglobin, hematocrit, numbers of erythrocytes, increased numbers of reticulocytes, polychromasia, poikilocytosis, anisocytosis -both sexes) and biochemical parameters (increased serum cholesterol levels - both sexes, phosphorous levels - males only). Macroscopic discolouration of the lungs, which persisted throughout the recovery period, was correlated with the presence of black pigment granules within alveolar macrophages of animals of all treatment groups. An increase in liver/brain weights (statistically significant only in the females), increased lung/trachea/body weight ratios and the presence of small cystic spaces containing basophilic mucoid material in the nasal cavity epithelium was still evident after the recovery period in both sexes.

Male and female rats of the low dose group were observed to have mild poikilocytosis and anisocytosis while the females only showed the occasional cyst of the epithelium in the nasal cavity. All hematological findings in low dose animals showed recovery.

Based on the results of this study, the systemic LOAEL is 49 mg/m ^{3} for both sexes, based on cardiac pathology, decreased body weight gain, altered hematology and clinical chemistry, and gross pathological findings in the lungs. The systemic NOAEL is 5.4 mg/m^3 (0.005 mg/L) for P1/P13.

This study is classified as **acceptable** (guideline) ans satisfies the guideline requirement (OPPTS 870.3465; OPP 82-4) for a subchronic inhalation toxicity study in rats.